Rabies Virus

(615) 262-6350

Introduction

The direct fluorescent antibody procedure to detect rabies virus in brain material is performed at the Tennessee Department of Health Laboratories (TDH) in Jackson, Nashville, and Knoxville. This testing service is available to state rabies control personnel, veterinarians licensed in the state of Tennessee, and any health care provider licensed by and practicing in Tennessee. The mouse inoculation procedure to detect rabies virus is available for limited use at the Nashville laboratory.

Specimen Acceptance Policy

Only animals that have potentially exposed a person, household pet, or livestock to rabies or animals of interest to rabies control personnel should be submitted. Exposure is defined as a bite or contamination of scratches, abrasions, open wounds, or mucous membranes with infectious saliva.

Dogs and cats are the only animals that should be kept alive and held 10 days for observation following a bite. Observation is of value because the period of time the virus can be excreted in the saliva prior to onset of signs can be predicted. It is known that dogs and cats can excrete rabies virus up to five days prior to onset of signs. The ten-day observation period for dogs and cats is twice that predicted time, allowing a margin of safety. If a dog or cat shows no clinical signs of rabies after 10 days of observation, it is safe to assume that the animal was not shedding rabies virus at the time of the bite. Conversely, if a dog or cat exhibits signs of rabies, it should be tested. Euthanize the animal and submit only the head to the laboratory for testing.

Unlike dogs and cats, the period of time that rabies virus can be excreted in the saliva of wild carnivores (such as, foxes, skunks, raccoons, bobcats) before the animal shows signs of rabies cannot be predicted and therefore an animal in this group should not be held for observation following a bite. If testing criteria have been met, these animals should be caught and euthanized immediately. Only the head should be sent to the laboratory for rabies virus detection. Do not destroy the brain.

Bats to be tested should be caught, euthanized, and submitted whole to the laboratory for rabies virus detection. Do not destroy the brain.

Specimen Collection

Brain tissue is examined for the detection of rabies infection in animals. Therefore, only the animal's head should be submitted for diagnostic purposes. For bats, the whole DEAD animal should be submitted. Animals should be euthanized in a manner that will not destroy the brain. The animal's neck should be severed at the midpoint between the base of the skull and the shoulders.

Specimen Identification

1. Complete **all** the information on Rabies Form PH-1584.

2. Label the outside of the specimen container with the type of animal (dog, cat, cow, etc.) and the tear strip number from the accompanying Rabies Form PH-1584. Secure the label with transparent tape.

Rabies Virus (Continued)

Shipment of Specimen

1. Packing and shipping specimens to the state public health laboratory requires personnel trained in current regulations. See section I-17 for the ASM guidelines published in November, 2003. Specimens must not be frozen, fixed in formalin, or shipped on dry ice.

2. Affix the mailing label (PH-0838), return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the outer container.

3. Ship the specimen by the fastest means possible to the TDH Laboratory in **Jackson, Knoxville,** or **Nashville**. Transport by the provider's personal courier is preferred, but shipment by commercial couriers is acceptable, if permitted.

4. **It is against US postal regulations to send this type of specimen through the mail**.

Reporting Procedure and Interpretation

Positive rabies test results will be reported immediately by telephone to the provider of the specimen and the State Rabies Control Officer or his representative. Unsatisfactory specimens will be reported to the provider by telephone only if significant human exposure has occurred from a high-risk animal species. All test results will be mailed to the provider. The health department of the county in which the animal specimen was obtained will be sent a report of all positive results. Results are available within 24 to 36 hours after receipt of the specimen except for those specimens received on Friday afternoon. A shortened test procedure will be performed on all specimens received on Friday afternoon and for which a human exposure is indicated. This shortened test procedure produces a "preliminary result" that will be conveyed by telephone on Friday afternoon to the provider of the specimen. The provider will be cautioned that the "preliminary result" is not the final result and that the final result will be available on the following Monday.

Results of Fluorescent Rabies Antibody Tests Are Reported
Rabies virus found by FRA (Fluorescent Rabies Antibody)
Rabies virus not found by FRA (Fluorescent Rabies Antibody)

The mouse inoculation test, if needed, may take as long as 28 days before a result is available.

Results of Mouse Inoculation Tests Are Reported
Rabies virus found by mouse inoculation.
Rabies virus not found by mouse inoculation.

Criteria for Unacceptable Specimens

1. The specimen is received without an accompanying Rabies Request Form, PH-1584.
2. The brain material has deteriorated or decayed to the extent that anatomical features of the brain are no longer distinguishable.
3. The brain has been mutilated to the extent that anatomical features (areas) of the brain can not be distinguished.
4. There is no brain evident in the head submitted.
5. The specimen is fixed in formalin.

NOTE: Frozen specimens are not necessarily unacceptable. Freezing and thawing of brain tissue is very damaging to the tissue. The acceptability of a frozen specimen will be decided once the specimen is thawed. Submitting a frozen specimen usually causes at least a one day delay for rabies testing.

Rabies Form PH-1584

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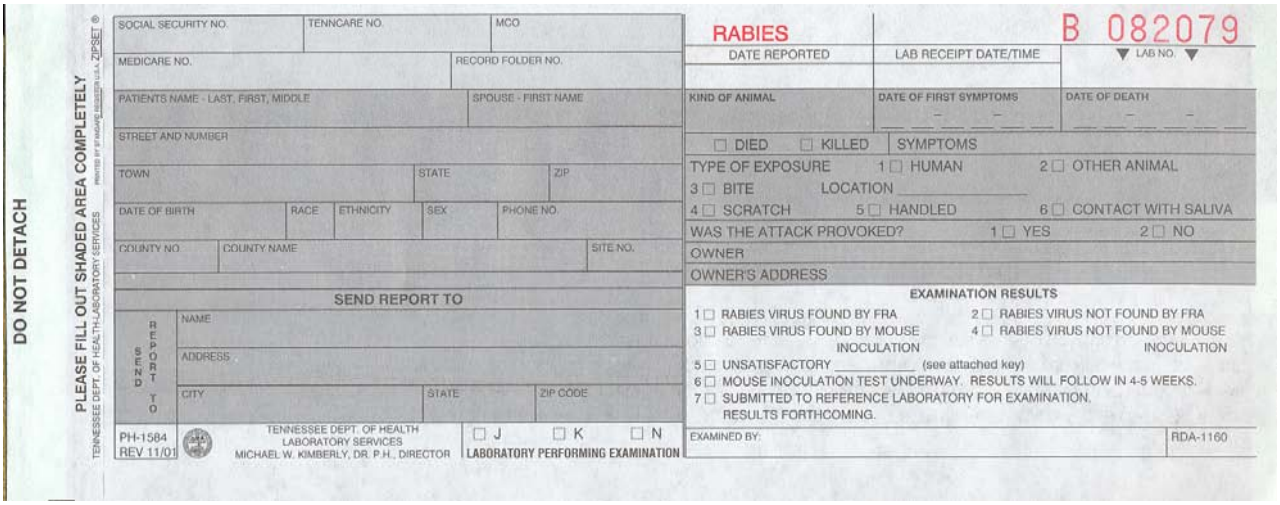


NOTHING ON BACK OF FORM

Virus Culture

(615) 262-6350

Introduction

The Virology Unit is responsible for culturing and identifying viruses in clinical specimens. Virus culture provides a mechanism for the detection and identification of many human viruses that cause a wide variety of common illnesses. Specimens for culture of human viruses will be accepted from both public and private health care providers.

Virus culturing and identification that is not performed at the Tennessee Department of Health (TDH) Laboratory may be available at the Centers for Disease Control and Prevention (CDC) in Atlanta. **Consult with the Virology Unit prior to submitting specimens for testing.** According to CDC's guidelines, all specimens submitted to the CDC must come through the state laboratory or receive the state laboratory's approval for direct shipment from the provider to the CDC.

Specimen Collection

Collect specimens for virus isolation during the early, acute, febrile phase of illness. Specimens collected more than one week after onset of symptoms usually do not yield live viruses. The source of the specimen collected must be carefully matched with the virus suspected. The virus isolation services available at the TDH Laboratory and the specimen of choice for each virus is described in Chart V - 3 VIRUSES FOR WHICH ROUTINE CULTURING IS AVAILABLE.

Collect the specimen aseptically and place it in one of the following environments immediately.

(1) Refrigerate at 2 to 8^{o}C if the specimen will be delivered to the laboratory within 48 hours.

(2) If receipt of the specimen by the laboratory will be longer than 48 hours from the time of collection, freeze the specimen at -70^{o}C or at the lowest temperature possible and ship to remain frozen during transport.

NOTE: DO NOT FREEZE THE FOLLOWING (Ship with "cold packs" (artificial refrigerant):

Specimens for isolation of respiratory syncytial virus (RSV).
Specimens for isolation of cytomegalovirus (CMV).
Blood specimens for virus isolation.

Autopsy or Biopsy: Collect fresh, unfixed tissue from the probable sites involved using a separate sterile instrument for each sample. Place each specimen into a separate small sterile vial of virus transport medium.

Consult with the Virology Unit prior to submitting autopsy or biopsy specimens for virus isolation.

Cerebrospinal Fluid (CSF): Aseptically collect 2 to 3 ml of CSF and transfer it to a sterile vial.

Feces: Place a piece of feces 4 to 8 grams (about the size of a grape) into a sterile container.

Rectal Swab: Generally, rectal swabs are less satisfactory than feces for the isolation of viruses. If used, obtain a rectal swab by inserting a dry cotton swab at least 5 cm into the anal orifice, rotating the stick, and then withdrawing it. Some fecal material must be visible on the cotton. Break the tip off into a vial of viral transport medium.

Throat Swab Vigorously rub both tonsils and the posterior wall of the pharynx with a dry, sterile, cotton or dacron swab. The swab should not touch the tongue or buccal mucosa. Break off the swab tip into a vial of virus transport medium.

Chart V - 3
Viruses for Which Routine Culturing is Available
Consult with the TDH Laboratory for additional tests
that may be available at the CDC.

Virus	TEST METHOD	SPECIMEN SOURCE/TYPE
Adenovirus (Types 1-41)	Cell culture FA Neutralization	Throat washing or swab, nasopharyngeal wash or swab, conjunctival swab, feces, urine
Coxsackie virus (A & B)	Cell culture Neutralization	Throat swab, feces, CSF, pericardial fluid
Cytomegalovirus	Cell culture FA	Urine, throat swab, buffy coat, lung tissue, lung aspirate
Echovirus (Types 1-33)	Cell culture Neutralization	Throat swab, feces, CSF, pericardial fluid
Enterovirus (Types 68-71)	Cell culture Neutralization	Throat swab, feces, CSF, pericardial fluid, vesicle scraping
Herpes simplex virus (Types 1 & 2)	Cell culture FA	Vesicle scraping, brain biopsy, conjunctival swab, urogenital swab
Influenza virus (A & B)	Cell culture FA HA HI	Throat washing or swab, nasopharyngeal washing or swab
Measles virus (Rubeola)	Cell culture FA	Throat washing or swab, nasopharyngeal washing or swab, conjunctival secretions
Mumps virus	Cell culture HAdI FA	Throat washing or swab, urine, CSF
Parainfluenza virus (Types 1,2,& 3)	Cell culture FA HAdI	Throat washing or swab, nasopharyngeal washing or swab
Poliovirus (Types 1,2,& 3)	Cell culture Neutralization	Throat washing or swab, feces, nasopharyngeal washing or swab, rectal swab
Respiratory syncytial virus	Cell culture FA	Nasopharyngeal washing or swab
Rubella virus	Cell culture FA	Nasopharyngeal washing or swab, CSF, urine
Varicella-zoster virus (Chickenpox-shingles)	DFA	Vesicle scraping, swabbing

Abbreviations
FA - Fluorescent antibody
DFA- Direct Fluorescent Antibody
HA - Hemagglutination
HI - Hemagglutination inhibition
HAdI - Hemadsorption inhibition

Virus Culture (Continued)

Throat Washing: The patient should gargle with approximately 8 ml of suitable washing fluid (such as, tryptose phosphate broth, Hank's balanced salt solution, veal infusion broth, or sucrose-phosphate buffer). Collect the fluid in a sterile container with a leak-proof top.

Nasal Swab: Insert a dry cotton or polyester (not alginate) swab into the nostril parallel to the palate and leave in place for a few seconds. Slowly withdraw it with a rotating motion. Obtain specimens from both nostrils with the same swab. Break off the tip of the swab into a tube containing approximately 1.5 ml of viral transport medium.

Nasal Washing: Place the patient in a comfortable position with the head slightly tilted backward. Advise him to keep the pharynx closed by saying "K" while the washing fluid is applied to the nostril. With a transfer pipette, apply 1 to 1.5 ml of washing fluid to one nostril at a time. Ask the patient to tilt his head forward and let the washing fluid flow into a sterile beaker or petri dish. Repeat the process alternately with both nostrils until approximately 8 ml of the washing fluid has been used. Transfer the washings from the sterile catch container (the sterile beaker or petri dish) to a sterile container with a leak-proof top for transport to the laboratory.

Nasopharyngeal Swab: Take nasal and throat swabs as described above and place into the same vial of transport medium.

Nasopharyngeal Washing: Take nasal and throat washings as described above and place into the same vial of transport medium.

Urine: Collect clean-catch urine, preferably the first voided morning urine, in a sterile container.

Vesicle: Using a sterile instrument, open the fluid-filled vesicle. Using firm pressure, absorb the fluid with a sterile cotton swab and scrape the perimeter of the lesion obtaining cellular material on the swab tip. Avoid causing excessive bleeding. Break off the swab tip into a vial of virus transport medium.

Tissue Culture Isolates: The Virology Unit provides reference services for laboratories throughout Tennessee that perform viral isolation. Viral isolates should be observed microscopically at the initial laboratory until 50% or more of the available cell sheet is exhibiting viral cytopathic effect (CPE). Once the cell sheet is exhibiting 50% CPE, send a tube of the infected cell culture (frozen or unfrozen) to the TDH Nashville Laboratory for identification and/or typing of the virus. If the specimen is to be transported at ambient temperature, the tube of infected cell culture should be filled with a cell-culture-maintenance medium. If the specimen is frozen for transport no more than 1 ml of maintenance medium should be in the tube. Indicate the type of cell culture and the number of times the virus was passed through culture on the specimen tube. Indicate the suspected virus on the form.

Specimen Identification

1. Complete **all** the information on the Virology Form PH-1579. Include pertinent clinical information with each specimen.

2. Using indelible ink, label each specimen with the patient's first and last name, the specimen source, and the date of collection. Attach the control number on the tear strip to the specimen with transparent tape. Unlabeled specimens or specimens that contain information that is not compatible with the information on the test request form, **will not be tested**.

Shipment of Specimens

1. Specimens that are to be hand-carried to the laboratory should be wrapped in paper toweling and placed in a leak-proof container with either ice or commercial refrigerant packs. Be careful not to contaminate the specimen with any of the moisture produced by the refrigerant and be careful not to break the specimen container by crushing or colliding with the refrigerant.

2. Specimens that will arrive at the testing laboratory more than 48 hours after collection should be frozen as soon after collection as possible. Pack them with enough dry ice to last 48 hours longer than the expected time required for transport of the specimen to the laboratory. Pack specimens so that direct contact with the dry ice is prevented. Place the form in or on the container so that the test request form cannot be contaminated by the specimen even if breakage of the primary specimen container should occur.

   Do not freeze specimens for isolation of respiratory syncytial virus (RSV) or cytomegalovirus (CMV) or blood specimens. Ship these specimens refrigerated.

3. Place the mailing label (PH-0838), return address, infectious substance (etiologic agent) or clinical (diagnostic) specimen label, and dry ice label on the shipping container.

4. Ship the specimen to the TDH Laboratory in **Nashville**.

5. Use first-class postage on US mail.

Reporting Procedures and Interpretation

Reporting of Results for Virus Cultures
Positive for _____. The name of the virus isolated. (The type is included if appropriate.)
No virus isolated.

Turn-around time for negative cultures varies from 1 to 4 weeks. Cultures yielding virus isolates may require more time for identification of the virus depending upon the isolate involved. Failure to isolate a virus may be the result of a number of factors, including improperly collected specimens, specimens collected at a period in the disease when the patient is not shedding virus, improperly transported specimens, or a lack of sensitivity in the system being used for isolation. Failure to isolate virus should not rule out the virus as a cause of the clinical illness. Conversely, since people may asymptomatically carry a variety of viruses, viruses may be isolated which are unrelated to the current clinical illness.

Virus Culture (Continued)

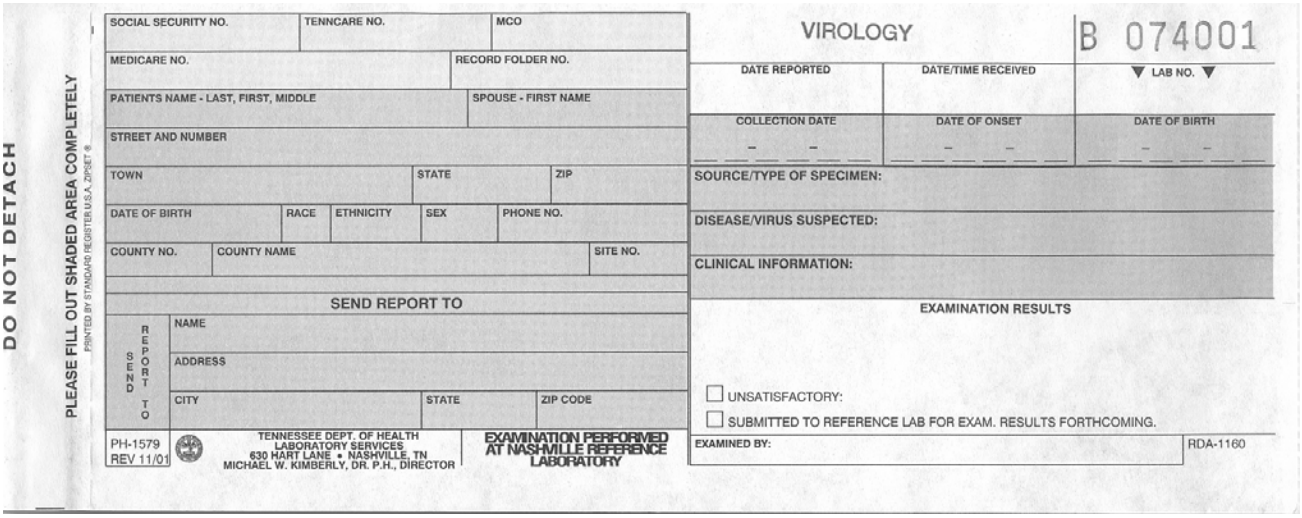
The results of all specimen requests are reported to the provider who submitted the specimen. In addition the TDH Communicable and Environmental Disease Services and the health department in the county where the patient lives are sent reports on all positive results.

Criteria for Unacceptable Specimens

1. The specimen is not properly identified with the patient's name.
2. The patient identifiers on the specimen do not exactly match those on the test request form.
3. The specimen is broken or leaked in transit.
4. The specimen is inappropriate for virus isolation.
5. The quantity of specimen received is not sufficient to perform the requested testing. (QNS - Quantity Not Sufficient.)
6. The specimen is received in a compromising condition (i.e., warm, delayed in transit) situation.

Virology Form PH-1579

FRONT



NOTE: VIROLOGY FORM HAS NO PRINTING ON BACK